

# **The phosphatidylinositol-transfer protein Nir3 promotes PI(4,5)P<sub>2</sub> replenishment in response to TCR signaling during T cell development and survival**

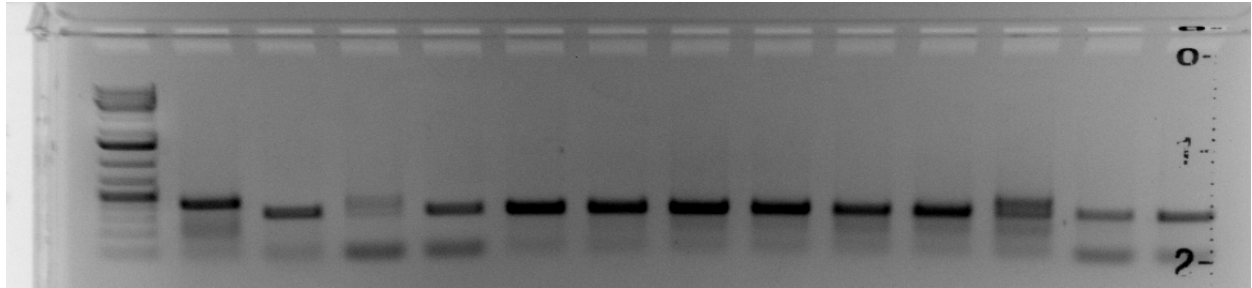
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**Supplementary Figure 1: Generation of *Nir3*<sup>-/-</sup> mice.** (a) *Nir3* is located on the 5<sup>th</sup> chromosome in mice. The translation start codon is located on the 3<sup>rd</sup> exon. A guide RNA (gRNA) targeting site on the 4<sup>th</sup> exon is underlined and the PAM sequence is marked in red. The gRNA was injected into the C57BL/6 zygotes with the purified Cas9 protein. The resulting pups carried a 98bp deletion on the 4<sup>th</sup> exon which led to a reading frame shift and introduced a stop codon. (b) *Nir3*<sup>-/-</sup> mice were confirmed with a primer pair amplifying a region flanking the targeted region. (c) Immunoblot of Nir3 levels from the thymocytes of WT and *Nir3*<sup>-/-</sup> mice. Actin was used as a loading control. Data are representative of at least 3 separate biological replicates.

Original gels and blots:

b



c

Long (left) and short (left) exposure (Upper half: Nir3, Lower half: actin)

